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Report

Dependence on the Lazaro Phosphatidic Acid Phosphatase for the Maximum Light Response

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Summary

The *Drosophila* phototransduction cascade serves as a paradigm for characterizing the regulation of sensory signaling and TRP channels in vivo [1, 2]. Activation of these channels requires phospholipase C (PLC) and may depend on subsequent production of diacylglycerol (DAG) and downstream metabolites [3, 4]. DAG could potentially be produced through a second pathway involving the combined activities of a phospholipase D (PLD) [5] and a phosphatidic acid (PA) phosphatase (PAP). However, a role for a PAP in the regulation of TRP channels has not been described. Here, we report the identification of a PAP, referred to as Lazaro (Laza). Mutations in *laza* caused a reduction in the light response and faster termination kinetics. Loss of *laza* suppressed the severity of the phenotype caused by mutation of the DAG kinase, RDGA [6, 7], indicating that Laza functions in opposition to RDGA. We also showed that the retinal degeneration resulting from overexpression of the PLD [5] was suppressed by elimination of Laza. These data demonstrate a requirement for a PLD/PAP-dependent pathway for achieving the maximal light response. The genetic interactions with both *rdgA* and *Pld* indicate that Laza functions in the convergence of both PLC- and PLD-coupled signaling in vivo.

Results and Discussion

To identify new proteins that are involved in phosphoinositide (PI) signaling and participate in *Drosophila* phototransduction, we focused on a gene (*CG11440*), which we previously identified in a microarray screen for genes expressed predominately in the adult eye [8]. *CG11440* mRNA is expressed in the adult eye at a concentration 15-fold higher than in the rest of the head and encodes a 334 amino acid protein that is >35% identical to known lipid phosphate phosphatases (reviewed in [9]). As with other lipid phosphate phosphatases (LPPs), *CG11440* is characterized by multiple transmembrane segments and three conserved domains that form the catalytic domain. An LPP would be predicted to catalyze the reverse reaction promoted by RDGA (Figure 1A).

To address a potential function for the *CG11440* (referred to as *lazaro*, *laza*; see figure legends for an explanation of the gene name) in phototransduction, we obtained a P element insertion line, GE27043 (GenExel), which was inserted in the 5′ untranslated region (*laza^{GE}*; Figure 1B). We created a null mutation in *laza* by genetically mobilizing the P element, because excisions of P elements are frequently associated with deletions of the flanking genomic DNA. We screened pools of genomic DNA by PCR and obtained two lines (*laza^{1}* and *laza^{2}*) that deleted the first ATG and additional portions of the coding region (Figure 1B). In addition, we retained one revertant line in which the P element excised precisely (*laza^{RE}*).

We raised antibodies against the C-terminal region of Laza and identified a protein of 42 kDa (predicted molecular weight of 37 kDa) that was not detected in any of the *laza* mutants (*laza^{GE}*, *laza^{1}*, *laza^{2}*) or in flies containing *laza^{GE}* in *trans* with a deficiency chromosome, *Df(3L)ED230*, that removed *laza* (Figures 2A and 2B). The 42 kDa protein was restored in *laza^{RE}* as well as in *laza^{1}* flies in which we introduced a wild-type *CG11440* genomic transgene (Figure 2B). These data indicated that expression of the Laza protein was disrupted in *laza^{GE}*, *laza^{1}*, and *laza^{2}*. Protein levels of other phototransduction proteins were unchanged in each of the lines studied (Figure 2C).

To evaluate whether the Laza protein was expressed in the retina, we used a biochemical approach because the antibodies were ineffective for immunostaining. We detected Laza exclusively in extracts from wild-type heads but not bodies (Figure 2D). Moreover, the Laza protein was absent in head extracts prepared from a mutant lacking eyes (*sine oculis*, *so*) and was greatly reduced in the heads of a mutant (*glass*), which were missing photoreceptor cells specifically [10] (Figure 2E). Thus, Laza was expressed primarily in photoreceptor cells.

We evaluated the requirement for *laza* for phototransduction by performing electroretinogram (ERG) recordings, which assay the summed responses of all retinal cells to light. Exposure of wild-type flies to light results in a corneal negative receptor potential, which decays to baseline after cessation of the light stimulus (Figure 3A; 10.3 ± 0.7 mV, n = 17). The amplitude of the light response was reduced nearly 40% in *laza^{GE}* flies (6.5 ± 0.4 mV, n = 29) and 3-fold in *laza^{1}* (3.7 ± 0.4 mV, n = 19) and *laza^{2}* flies (2.8 ± 0.3 mV, n = 23) (Figures 3B–3D and 3H). These reductions in ERG amplitudes were not due to background mutations because they were observed in *laza^{GE}/Df* flies (Figures 3E and 3H; 5.9 ± 0.3 mV, n = 25) and the *laza^{GE}* phenotype reverted to wild-type upon precise excision of the P element (Figures 3F and 3H; 10.1 ± 0.2 mV, n = 19). Furthermore, the wild-type *laza^{+}* transgene rescued the reduction in the ERG amplitude (Figures 3G–3H; 10.9 ± 0.3 mV, n = 12). Although it was difficult to formally exclude that the *laza* mutation had no effects on the overall physiology of the photoreceptor cells, the reduced ERG amplitude in *laza* flies did not appear to be due to retinal degeneration because 7-day-old adults did not display retinal degeneration (Figure S1 in the Supplemental Data

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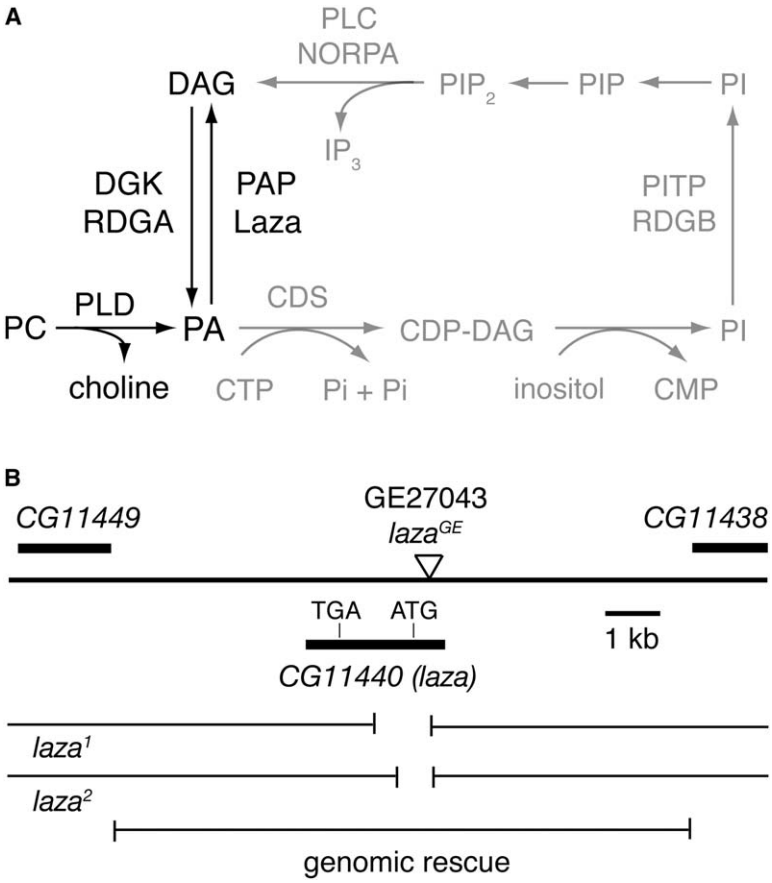

Figure 1. Phosphoinositide Signaling and the *laza* Genomic Region

(A) PI cycle. Activation of PLC (NORPA), which occurs following light stimulus, leads to hydrolysis of PIP_2 and generation of DAG and IP_3. The PIP_2 is regenerated through a multistep cycle initiated with the phosphorylation of DAG by the DAG kinase (RDGA) to produce PA. The proposed site of action of Laza is indicated, and it is in opposition to RDGA. PA is also produced from PC through the action of PLD. The following abbreviations are used: CDS, CDP-diacylglycerol synthase; DAG, diacylglycerol; DGK, DAG kinase; IP_3, inositol 1,4,5-trisphosphate; Laza, Lazaro; NORPA, No Receptor Potential A; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PC, phosphatidylcholine; PI, phosphatidylinositol; PITP, PI-transfer protein; PIP, phosphatidylinositol-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; PLD, phospholipase D; RDGA, Retinal Degeneration A; and RDGB, Retinal Degeneration B.

(B) Schematic representation of the *laza* mutations. A P element insertion (GE27043) was used to generate the deletions in $laza^1$ and $laza^2$ through imprecise excision of the transposable element. The 1.1 and 0.6 kb deletions in $laza^1$ (−317 to +1381) and $laza^2$ (−309 to +913), respectively, are indicated.

available online) and the ERGs were performed on flies <7 days old. As is the consequence of most mutations that affect phototransduction, in older flies (≥15 days old) the *laza* mutation resulted in retinal degeneration, which was light-dependent (data not shown). Nevertheless, the decreased ERG responsiveness in the *laza* flies was not age dependent (Figure 3I).

To assess whether the decreased amplitude in *laza* was more likely a reflection of a lower sensitivity to light or a decreased maximal obtainable amplitude, we measured the ERG responses at three different intensities. Over the 130-fold intensity range tested, both wild-type and *laza* flies showed a linear intensity-responsive relationship when the amplitudes were plotted against the logs of the light intensities (Figure 3J). However, in the *laza* mutant, the plot is shifted down. If there was a reduction strictly in the sensitivity of the light response, the lines generated from the wild-type and *laza* intensity-responsive relationships should have been parallel. This was not the case, because the amplitude of the *laza* response increased less sharply than in wild-type (Figure 3J). These data are most consistent with a defect in the maximum light response in the mutant; however, the data do not allow us to assess whether or not there is a defect in the sensitivity to light. If *laza* operates in a pathway downstream of PLD (Figure 1A), then there would be expected to be overlaps in the *pld* and *laza* mutant phenotypes. Interestingly, in the *pld* null mutant, there is also a defect in the maximum achievable ERG amplitude [5].

Mutations in *rdgA*, which encodes a diacylglycerol (DAG) kinase [11], prolong termination kinetics [4]. To test whether elimination of *laza* might accelerate deactivation kinetics, consistent with the predicted biochemical function (Figure 1A), we assayed the time required for an 80% return to the baseline after cessation of the photoresponse. We found that the termination of the photoresponse was approximately 4-fold faster in the $laza^1$ (0.29 ± 0.02 s, n = 19) and $laza^2$ (0.25 ± 0.03 s, n = 18) mutant flies than in wild-type (1.24 ± 0.11 s, n = 20) (Figures 4A–4C and 4G). Introduction of the genomic rescue transgene in the $laza^1$ mutant background restored the deactivation kinetics to that of wild-type (Figures 4D and 4G; 1.29 ± 0.11 s, n = 16).

The apparent increase in the speed of termination of the photoresponse in the *laza* mutants may have been due to the smaller ERG amplitude. Therefore, we exposed wild-type flies to a lower light intensity so that the amplitudes of the wild-type and *laza* ERGs were comparable (Figures 4B, 4C, and 4E). Under these conditions, the time required for 80% termination of the photoresponse was 2-fold shorter in the $laza^1$ (0.29 ± 0.02 s, n = 19) and $laza^2$ (0.25 ± 0.03 s, n = 18) mutant flies than in wild-type (0.53 ± 0.04 s, n = 20) (Figures 4B, 4C, 4E, and 4H). Thus, the more rapid termination in *laza* flies was the reverse of that observed in *rdgA* flies.

If *laza* functions in the regulation of the photoresponse by catalyzing the PI-cycle reaction that is the reciprocal of that catalyzed by RDGA (Figure 1A), then it is plausible that introduction of *laza* into an *rdgA* background would

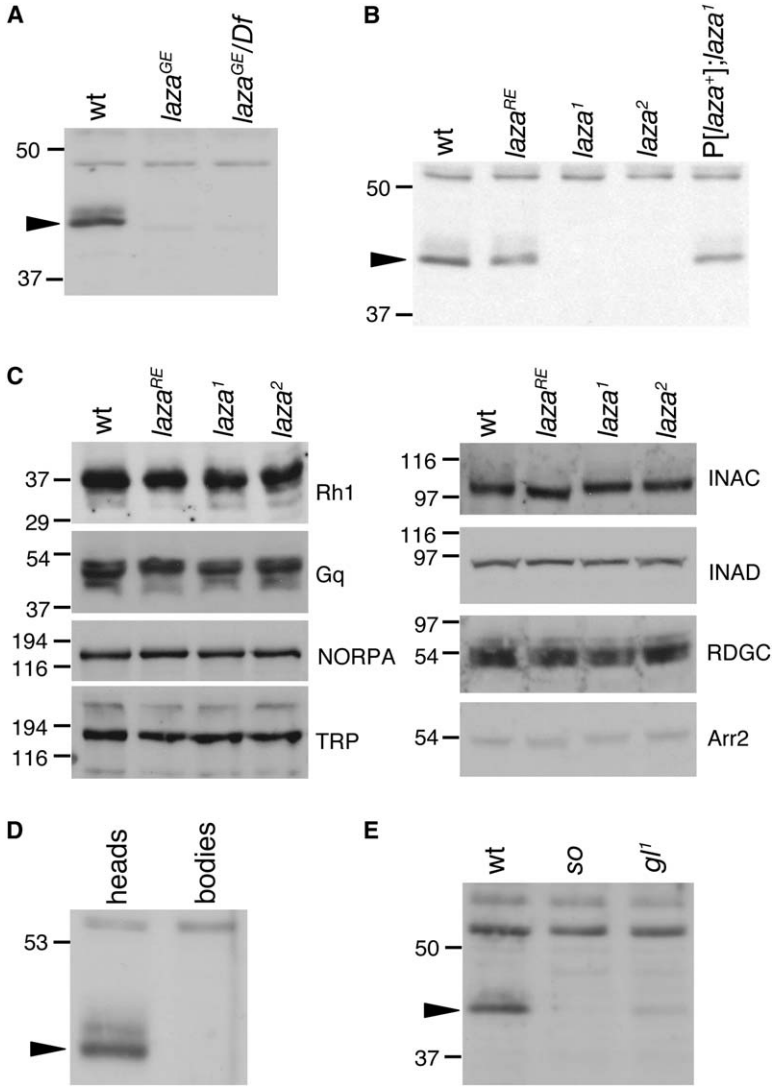

Figure 2. Analysis of Laza Protein Expression on Western Blots

(A) Reduction of Laza expression in *laza*^{GE} flies. To perform the western blots, we prepared head extracts in PBS from the following fly stocks, added 1% Triton X-100 to the pellets, fractionated the supernatants by SDS-PAGE, and probed the blots with anti-Laza antibodies: (1) wild-type (wt), (2) *laza*^{GE}, and (3) *laza*^{GE}*/Df(3L)ED230*. The 42 kDa Laza protein is indicated by the arrowhead. The higher-molecular-weight band is nonspecifically recognized by the antibodies. This additional band serves as a loading control.

(B) Restoration of Laza expression in flies that have precisely excised the P element (*laza*^{RE}) and in *laza*^{1} containing the wild-type *laza*$^{+}$ transgene (P[*laza*$^{+}$];*laza*^{1}).

(C) The protein levels of other signaling molecules are not changed in *laza*. Western blots of whole head extracts prepared from wild-type, *laza*^{GE}, *laza*^{1}, and *laza*^{2} were probed with antibodies to the indicated proteins.

(D) Laza expression is detected in wild-type heads but not bodies.

(E) Laza expression is reduced in heads of mutants missing eyes (*sine oculis*, *so*) or photoreceptor cells (*glass*^{1}).

suppress the retinal degeneration observed in *rdgA* flies. To test this hypothesis, we combined the *laza*^{1} mutation with the *rdgA*^{BS12} mutation and tested for suppression of the retinal degeneration. Wild-type flies contain compound eyes consisting of ~800 repetitive units, referred to ommatidia, each of which contains seven photoreceptor cells in any given cross-section [1, 12]. Each photoreceptor cell includes a microvillar structure, the rhabdomere, which is the site of action of phototransduction. Wild-type flies contain seven rhabdomeres in all ommatidial cross-sections regardless of their age [1, 12]. In contrast, the number and size of the rhabdomeres is dramatically reduced even in 1-day-old *rdgA* flies as a result of constitutive activation of the TRP channel [4] (Figures 5A–5C; 2.7 ± 0.13 rhabdomeres/ommatidia; 13.2 ± 1.1% cross-sectional area relative to wild-type).

Of significance here, the *laza*^{1} mutation suppressed the degeneration-associated *rdgA*^{BS12}. At 1 day posteclosion, the average number and size of the rhabdomeres was significantly increased (Figures 5A–5C; 5.7 ± 0.07 rhabdomeres/ommatidia; 42.8 ± 4.1% cross-sectional area relative to wild-type). By 3 days posteclosion, the double-mutant flies retained at least as many rhabdomeres as 1-day-old *rdgA*^{BS12} flies (Figure 5B). However, by 7 days after eclosion, the *rdgA*^{BS12}*;;laza*^{1} flies also displayed severe retinal degeneration (Figure 5B). Consistent with the partial suppression of retinal degeneration by the *laza* mutation, the ERG response in the double-mutant flies was also partially restored. Whereas there was virtually no light response in 1-day-old *rdgA*^{BS12} flies (0.10 ± 0.05 mV, n = 16), a small ERG was restored in the *rdgA*^{BS12}*;;laza*^{1} flies (0.64 ± 13 mV, n = 21) (Figures 5D and 5E–H). The suppression of *rdgA* may not have been complete because of the presence of seven other PAPs encoded in the *Drosophila* genome, including *wunen*, *wunen 2* [13–16], *CG11425*, *CG11426*, *CG11437*, *CG11438*, and *CG12746*, which could potentially be expressed in the photoreceptor cells.

Overexpression of phospholipase D (PLD) causes light-dependent retinal degeneration that is suppressed by mutation of the TRP channel [5]. Because PLD converts phosphatidylcholine (PC) to PA, the retinal degeneration could result from an elevation in phosphatidylinositol 4,5-bisphosphate (PIP_2) production (Figure 1A). Alternatively, an increase in production of PA could

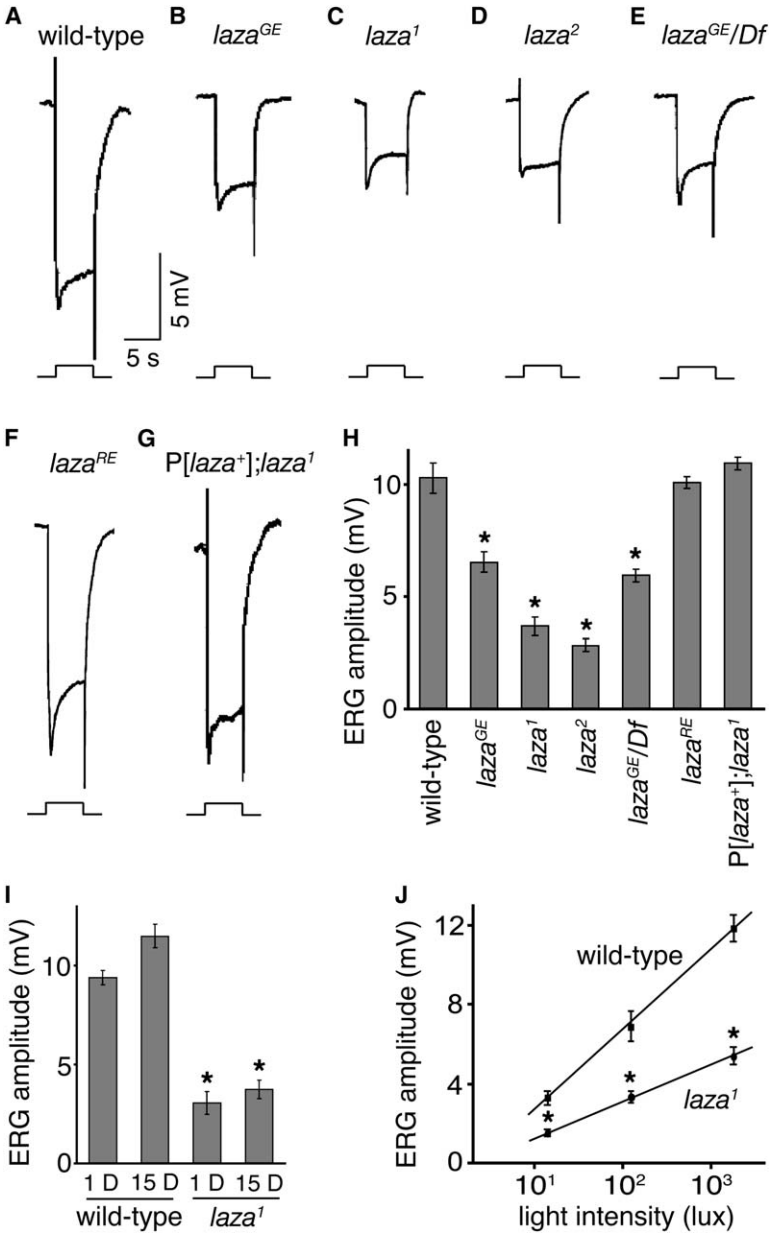

Figure 3. Small ERG Amplitudes in *laza* Mutants

(A–G) ERG recordings in response to a 5 s pulse of orange light (1970 lux). The recordings were performed essentially as described [24]. The light pulses are indicated by the event markers below the traces. (A) shows wild-type, (B) $laza^{GE}$, (C) $laza^{1}$, (D) $laza^{2}$, (E) $laza^{GE}/Df(3L)ED230$, (F) $laza^{RE}$, and (G) P[$laza^{+}$]; $laza^{1}$.

(H) Quantification of ERG amplitudes. The average amplitudes (mV), standard errors of the mean (SEMs), and number of flies examined (n) were as follows: wild-type (10.3 ± 0.7, n = 17), $laza^{GE}$ (6.5 ± 0.4, n = 29), $laza^{1}$ (3.7 ± 0.4, n = 19), $laza^{2}$ (2.8 ± 0.3, n = 23), $laza^{GE}/Df$ (5.9 ± 0.3, n = 25), $laza^{RE}$ (10.1 ± 0.2, n = 19), and P[$laza^{+}$];$laza^{1}$ (10.9 ± 0.3, n = 12). Statistically significant differences from wild-type ERGs were assessed with the unpaired student's t test. An asterisk indicates a value <0.05.

(I) The reduction of the ERG amplitude in *laza* was not age dependent. Shown are the average ERG amplitudes (mV ± SEMs) in 1- and 15-day-old wild-type and $laza^{1}$ flies: wild-type 1 day (9.4 ± 0.4; n = 11), wild-type 15 day (11.5 ± 0.6; n = 11), $laza^{1}$ 1 day (3.1 ± 0.6; n = 13), and $laza^{1}$ 15 day (3.8 ± 5; n = 12).

(J) Light-intensity response relationships in wild-type and *laza*. Shown are the average ERG amplitudes as a function of the log of the light intensity (mV ± SEMs): wild-type (14 lux, 3.3 ± 0.3; 124 lux, 6.9 ± 0.8; 1824 lux, 11.9 ± 0.7; n ≥ 13) and $laza^{1}$ (14 lux, 1.5 ± 0.2; 124 lux, 3.3 ± 0.3; 1824 lux, 5.4 ± 0.4; n ≥ 13). Asterisks indicate statistically significant differences (unpaired student's t test value <0.05).

lead to higher DAG levels, an increase that causes degeneration as a result of higher levels of TRP channel activation. Indeed, it has been shown in mammalian cell culture systems that DAG can be generated from PC by the sequential reactions promoted by PLD and a PA phosphatase [17]. These observations raise the interesting possibility that Laza may function in a common pathway with the PLD. To test this idea, we assessed whether the retinal degeneration caused by overexpression of PLD [5] was suppressed by the *laza* mutation. One-day-old *Pld*-overexpression flies (*Rh1-Pld*) displayed early stages of degeneration and were typically missing one rhabdomere per ommatidium (Figure 5G; 6.4 ± 0.03 rhabdomeres/ommatidium) [5]. By two days posteclosion, the *Pld*-overexpression flies displayed clear retinal degeneration; the numbers and sizes of the rhabdomeres were greatly reduced (Figures 5F–5H; 2.7 ± 0.3 rhabdomeres/ommatidia; 16.2 ± 1.1% cross-sectional area relative to wild-type). The degeneration gradually progressed until nearly all of the rhabdomeres disappeared in 7-day-old *Rh1-Pld* flies (Figure 5G). In contrast, no degeneration was detected in 1-day-old *Rh1-Pld;laza*^{1} flies, and the retinal degeneration was significantly reduced in the 2-day-old *Rh1-Pld;laza*^{1} relative to the *Pld*-overexpression flies (Figures 5F–5H; 5.2 ± 0.4 rhabdomeres/ommatidium; 36.4 ± 2.7% cross-sectional area relative to wild-type). Suppression was detected in 3-day-old flies (Figure 5G; rhabdomeres/ommatidium: Rh1-Pld, 1.9 ± 0.1; *Rh1-Pld;laza*^{1}, 3.3 ± 0.2); however, by 7 days posteclosion, the differences between *Rh1-Pld* and *Rh1-Pld;laza*^{1} were not significant (Figure 5G). These data indicated that the severity of the retinal degeneration in *Rh1-Pld* flies was delayed by introduction of the $laza^{1}$ mutation.

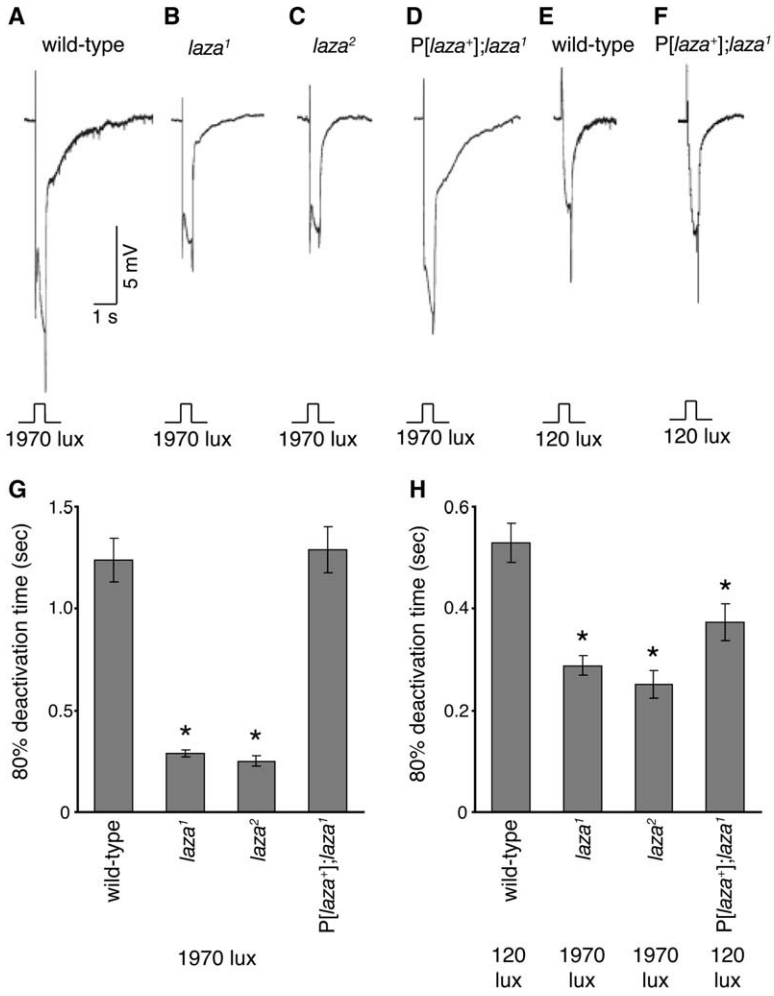

Figure 4. Decreased Deactivation Time in *laza*^{1} and *laza*^{2}

Flies were exposed to a short (0.5 s) pulse of orange light, which we have previously used to assay deactivation kinetics of other mutants [25, 26]. The intensities of the light used are indicated (1970 lux or 120 lux). The time required for an 80% decline in the ERG after cessation of the light stimulus (80% deactivation time) was determined. (A–F) Representative ERGs showing deactivation kinetics. The light pulses are indicated by the event markers below the traces. (A) wild-type, 1970 lux. (B) *laza*^{1}, 1970 lux. (C) *laza*^{2}, 1970 lux. (D) P[*laza*$^+$];*laza*^{1}, 1970 lux. (E) wild-type, 120 lux. (F) P[*laza*$^+$];*laza*^{1}, 120 lux.

(G) Average 80% deactivation times after stimulation with the high intensity of light (1970 lux). The average time (sec), SEMs, and number of flies examined (n) were as follows: wild-type (1.24 ± 0.11, n = 20), *laza*^{1} (0.29 ±0.02, n=19), *laza*^{2} (0.25 ± 0.03, n = 18), and P[*laza*$^+$];*laza*^{1} (1.29 ± 0.11, n = 16). Statistically significant differences from wild-type were assessed with the unpaired student's t test. An asterisk indicates a value <0.05.

(H) Average 80% deactivation times in flies with similar ERG amplitudes. To achieve comparable amplitudes in wild-type and *laza* flies, we exposed the wild-type and P[*laza*$^+$];*laza*^{1} flies to 120 lux, whereas the *laza*^{1} and *laza*^{2} flies were exposed to 1970 lux. The *laza*^{1} and *laza*^{2} data are the same as in (G) and included again for comparison. The average time (sec), SEMs, and number of flies examined (n) were as follows: wild-type (0.53 ± 0.04 s, n = 20) and P[*laza*$^+$];*laza*^{1} (0.37 ± 0.04 s, n = 16). Statistically significant differences with wild-type (unpaired student's t test value <0.05) are indicated with asterisks. The P[*laza*$^+$];*laza*^{1} flies showed a deactivation time intermediate between wild-type and *laza*^{1} and *laza*^{2}.

The observation that mutations in *laza* suppress the degeneration resulting from loss-of-function mutations in *rdgA* provides genetic evidence that Laza functions opposite to RDGA in the phosphoinositide cycle, consistent with its predicted biochemical function. Furthermore, the faster termination kinetics in *laza* is the converse of that reported in *rdgA* mutant flies. These results suggest that the slower response kinetics in wild-type flies is due to Laza-dependent reduction in PA and production of DAG. The DAG or downstream metabolites, such as polyunsaturated fatty acids (PUFAs), may in turn slow down the termination kinetics as a result of prolonged activation of the channels. Several mammalian TRPC channels are activated by DAG [18–20], and *Drosophila* TRP and TRPL have been reported to be activated by PUFAs [3]. The findings in the current report support the model that DAG or downstream metabolites, such as PUFAs, activate the TRPC channels in fly photoreceptor cells. These include the smaller ERG responses and faster termination kinetics in *laza* mutant flies. Interestingly, a TRPC-like current in rabbit artery myocytes is suppressed by inhibitors of either PLD or PA phosphatases [21].

PA is generated in fly photoreceptor cells from two sources. The first involves the well-established PLC-dependent hydrolysis of the PIP_2 to produce DAG and phosphorylation by DAG kinase [22]. The second is cleavage of PC to generate PA through activity of PLD [5]. Overexpression of PLD results in retinal degeneration, and it has been proposed that this degeneration results from the subsequent conversion of excess PA to DAG, which in turn leads to overstimulation of the TRPC channels [5]. An indication that Laza may function in a PLD-mediated pathway leading to production of DAG is that the phenotypes resulting from loss-of-function mutations in either the *pld* [5] or *laza* are similar. In both cases, there is reduced responsiveness to light. More direct genetic support that Laza functions in a PC/PA/DAG pathway is that elimination of Laza suppresses the retinal degeneration resulting from overexpression of PLD. However, we cannot exclude that the PLD-dependent production of PA also couples to an alternative Laza-independent pathway.

We propose that the DAG formed through the PLD/Laza pathway primes the phototransduction cascade to increase the responsiveness to light. Given the prior

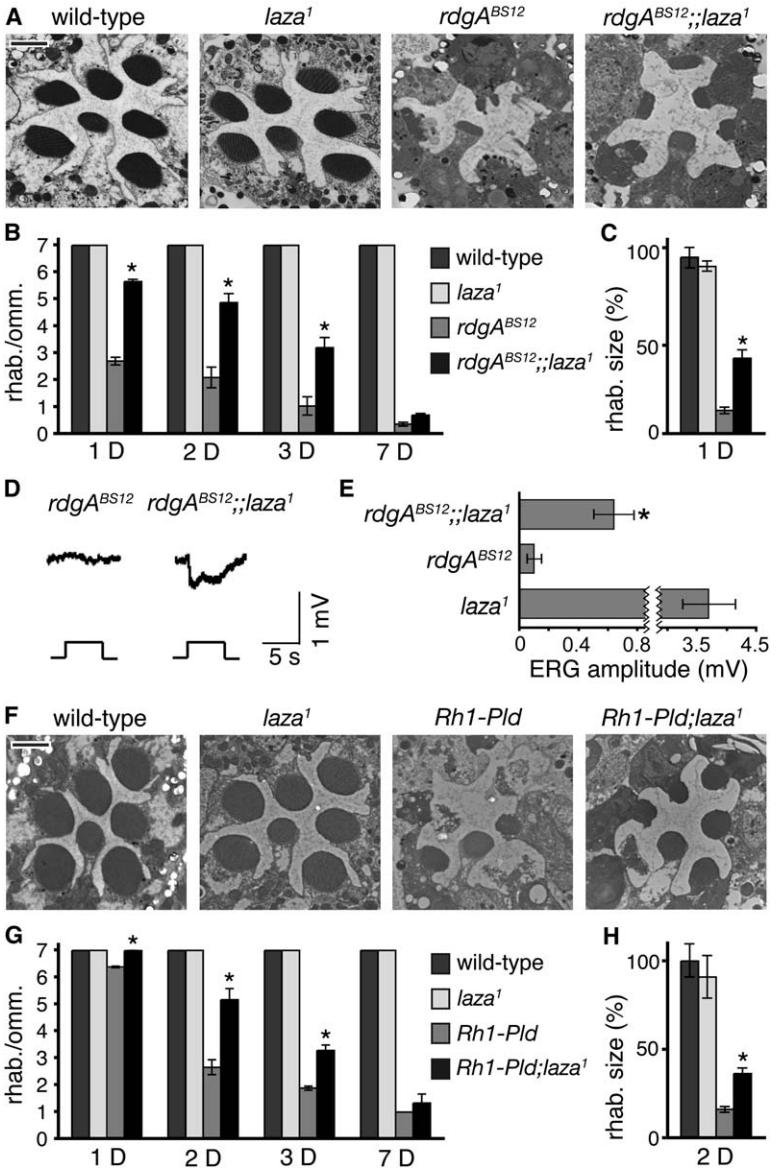

Figure 5. Genetic Interactions between *laza* and Two Other Genes that Function in the PI Cycle: *rdgA* and *Pld*

(A–E) Suppression of the retinal degeneration in $rdgA^{BS12}$ by elimination of $laza^{1}$. (A) Transmission EM images of single ommatidia from 1-day-old flies. The cross-sections were from the distal region of compound eyes embedded in LR White as described [27]. The scale bar represents 2 μm. (B) Average numbers of rhabdomeres per ommatidium. Quantification is based on examination of low-magnification EM and light-microscopy images of compound eyes. The average numbers of rhabdomeres per ommatidium ± SEM (at the indicated times in days posteclosion; D) were as follows ($n \geq 30$ ommatidia from 3 flies for all data): (1) wild-type (for all time points: 7 ± 0), (2) $laza^{1}$ (for all time points: 7 ± 0), (3) $rdgA^{BS12}$ (1 D, 2.7 ± 0.1; 2 D, 2.1 ± 0.4; 3 D, 1.0 ± 0.3; 7 D, 0.4 ± 0.07), and (4) $rdgA;;laza^{1}$ (1 D, 5.7 ± 0.1; 2 D, 4.9 ± 0.3; 3 D, 3.2 ± 0.4; 7 D, 0.7 ± 0.06). The asterisks in this and the following panels (C) and (E) indicate statistically significant differences between $rdgA^{BS12}$ and $rdgA^{BS12};;laza^{1}$ (<0.05) with the unpaired student's t test. (C) Relative rhabdomere sizes from 1-day-old wild-type, $laza^{1}$, $rdgA^{BS12}$, and $rdgA^{BS12};;laza^{1}$. Quantifications were based on examination of EM images of compound eyes (see Supplemental Experimental Procedures). The average size for wild-type is defined as 100%. Percentages ± SEM were as follows: (1) wild-type (100 ± 5.4), (2) $laza^{1}$ (94.7 ± 2.5), (3) $rdgA^{BS12}$ (13.2 ± 1.1), and (4) $rdgA^{BS12};;laza^{1}$ (42.8 ±4.1). (D) Partial rescue of the ERG phenotype in $rdgA^{BS12}$ by elimination of *laza*. Shown are ERGs obtained from 1-day-old $rdgA^{BS12}$ and $rdgA^{BS12};;laza^{1}$ with 1660 lux. Because the *laza* mutation partially restores a visual response to *rdgA* flies, the gene is referred to as *lazaro* (*laza*), a name based on the novel Lazarillo de Tormes, in which the orphan boy Lázaro helps a blind man see. (E) Quantification of ERG amplitudes (mV ± SEMs) with 1660 lux was as follows: $rdgA^{BS12};;laza^{1}$ (0.64 ± 0.13, n = 21), $rdgA^{BS12}$ (0.10 ± 0.05, n = 16), and $laza^{1}$ (3.7 ± 0.4, n = 19).

(F–H) Suppression of the retinal degeneration resulting from overexpression of PLD (*Rh1-Pld*) by the $laza^{1}$ mutation.

(F) Transmission EM images of cross-sections of ommatidia from 2-day-old flies. The scale bar represents 2 μm.

(G) The average numbers of rhabdomeres per ommatidium ± SEM (at the indicated number of days posteclosion ($n \geq 30$ ommatidia from 3 flies for all data) were as follows: (1) wild-type (for all time points: 7 ± 0), (2) $laza^{1}$ (for all time points: 7 ± 0), (3) *Rh1-Pld* (1 D, 6.4 ± 0.03; 2 D, 2.7 ± 0.3; 3 D, 1.9 ± 0.1; 7 D, 1.0 ± 0), and (4) $Rh1\text{-}Pld;laza^{1}$ (1 D, 7.0 ± 0; 2 D, 5.2 ± 0.4; 3 D, 3.3 ± 0.2; 7 D, 1.3 ± 0.3). The asterisks indicate statistically significance differences between *Rh1-Pld* and $Rh1\text{-}Pld;laza^{1}$ at a given time point (unpaired student's t test value <0.05).

(H) Relative rhabdomere sizes in 2-day-old flies. Percentages ± SEM were as follows: (1) wild-type (100 ± 8.8), (2) $laza^{1}$ (90.9 ± 11.7), (3) *Rh1-Pld* (16.2 ± 1.1), and (4) $Rh1\text{-}Pld;laza^{1}$ (36.4 ± 2.7). The asterisk indicates a statistically significant difference in rhabdomere sizes between *Rh1-Pld* and $Rh1\text{-}Pld;laza^{1}$.

evidence that PUFAs, which are formed from DAG, lead to activation of TRP channels [3, 4], the additional DAG formed through activity of Laza may be necessary for a maximal light response. We do not know whether or not Laza is expressed in the rhabdomeres or cell bodies; however, it is noteworthy that the PLD and RDGA are both in the cell bodies [5, 23], indicating that lipid metabolism in the cell bodies contributes to the photoresponse. The activity of the PLD has been suggested to be light independent [5]. Nevertheless, given that no light response is observed in the *norpA* mutant, we cannot exclude that PLD activity may be indirectly linked to the activity of *norpA*. It will be of interest to address the nature of the signals that contribute to PLD activation in vivo.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and one figure and are available with this article online at: http://www.current-biology.com/cgi/content/full/16/7/723/DC1/.

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